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14. ABSTRACT

We enrolled a total of 96 participants and completed data acquisition for 89 participants (53 with ASD and 36 controls). After performing qualitative and quantitative quality control and pre-processing of the data, we have been actively processing and analyzing the rich dataset from multiple perspectives. This includes investigation of how altered functional and structural connectivity in individuals with ASD is related to restrictive, repetitive behaviors and language abilities. We presented our preliminary findings at the Conference on Resting State Brain Connectivity (Sept 2014) and the Simons Center Annual Poster Session on the Social Brain (Nov 2014). Using data from this project as pilot, we have secured funding from the Simons Center for the Social Brain, the Bertarelli Foundation, and the NIH - Autism Center for Excellence Project to actively continue our work in autism.

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Table of Contents

INTRODUCTION:

This study utilized state-of-the-art complementary neuroimaging techniques to test the hypothesis that restricted, repetitive behaviors (RRBs) are associated with specific reductions in brain functional and structural connectivity, and to identify genetic contributions. The primary objective of this study is to illuminate the neural basis of RRBs in autism spectrum disorder (ASD) and its relation to specific genes. Identification of quantitative trait genetic loci that influence serotonin neurotransmission, mediate connectivity in specific neural circuitry, and are related to RRBs will clarify the pathophysiology of this disabling core feature of ASD and could lead to the use of genomics to individualize pharmacotherapy. Thus, this work will have a direct impact on both research and clinical care.

KEYWORDS

Autism Spectrum Disorder, structural connectivity, functional connectivity, resting-state, restrictive repetitive behaviors, speech, language networks, genetics, MRI, Diffusion Tensor Imaging

OVERALL PROJECT SUMMARY

Our Statement of Work indicated that during Year 03 of our 3-year study, our goal would be to enroll 12 control participants and 28 participants with ASD who meet our criteria. We would then acquire anatomical, DTI, and fMRI images as well as carry out genotyping on these 40 participants. We also indicated in our Statement of Work that we would be devoted to data processing, data analysis, manuscript preparation, and apply to other grants, if warrantied.

During the period of 09/06/2013-09/05/2014, we enrolled an additional 10 participants with ASD and 12 control participants. All of these participants completed the study and we were able to acquire phenotyping data, ASD characterization (if applicable), anatomical images, DTI images, fMRI images, and genotyping data.

We enrolled a total of 96 participants. Of these, 89 successfully completed all parts of this study. Phenotyping data have been entered in REDCap, a free, secure, web-based application designed to support data capture for research studies. Participants' saliva samples have been submitted to the Psychiatric & Neurodevelopmental Genetics Unit (PNGU) at MGH for preliminary processing and storage. We have also preprocessed the structural MRI, resting state bold, and DTI data. They are now ready for analysis.

Due to the significant effects of motion on functional and diffusion results, we are taking extra measures to perform quality control on our data, both by checking data during the scanning session and through post-hoc qualitative visual checks and quantitative analyses. For the functional data, we are using Dr. Susan Whitfield- Gabrieli's Artifact Detection Tools (ART) for automatic and manual detection of global mean and motion outliers in our fMRI data. ART allows us to remove time-series that have been affected by motion artifacts. For the diffusion data, we are using DTIPrep for automatic and manual removal of diffusion gradients that have been affected by motion, noise/SNR issues, vibrational artifact, venetian blind artifacts, etc. We also quantified both relative and absolute motion (in all translation and rotation directions) of our functional and diffusion data to verify that our ASD and control groups do not have significant differences in motion. We are integrating the data that have gone through ART and DTIPrep into our functional connectivity MRI (fcMRI) and DTI analysis pipelines. For DTI analysis, we are using Dr. Anastasia Yendiki's TRACULA (TRActs Constrained by Underlying Anatomy) for automatic reconstruction of a set of major white-matter pathways using global probabilistic tractography with anatomical priors.

Since anatomical and resting-state functional is not constrained by a task, it lends itself to numerous investigations. The following analyses projects are now underway:

- Functional and structural connectivity of the dorsal anterior cingulate cortex (dACC) in relation to RRBs in ASD
- Lateralization of the language and attention networks in ASD and its relation to cerebellar asymmetry
- Structural and functional connectivity of the speech and language networks in individuals with ASD using a comprehensive neurocomputational model of speech acquisition and production
- Identifying and characterizing the genetic basis of restrictive repetitive behaviors in ASD
- Dynamic functional connectivity in ASD using a data-driven ICA approach
- Effects of head motion on functional connectivity and diffusion data

We presented our initial findings on the altered resting state functional connectivity of the dACC in relation to RRBs at the Conference on Resting State Brain Connectivity (Sept 2014) and the Simons Center Annual Poster Session on the Social Brain (Nov 2014):

Title: Functional Connectivity of the Dorsal Anterior Cingulate Cortex Predicts Restrictive Repetitive Behaviors in Autism Spectrum Disorder.

Authors: T.Q.Nguyen, B. Baran, K.R. Van Dijk, S. Santangelo, S. Whitfield-Gabrieli, D.S. Manoach.

Abstract: Although restricted, repetitive behaviors (RRBs) are a highly disabling core feature of Autism Spectrum Disorders (ASDs), they have received little research attention and effective treatments are lacking. RRBs manifest as early as 18 months, predict outcome independently of social and communication deficits, and may interfere with the development of social and communication skills. Since converging lines of evidence have linked RRBs to the structure and function of the dorsal anterior cingulate cortex (dACC), the objective of the present study is to

investigate the functional connectivity of the dACC in individuals with ASD with a well-matched sample of neurotypical controls (NT). We show that aberrant resting-state functional connectivity of the dACC cognitive control network is associated with severity of the RRBs in ASD.

We are performing further analyses and preparing a manuscript for this work.

Using the data from this project as pilot, we have successfully secured funding from the Simons Center for the Social Brain, the Bertarelli Foundation, and the NIH - Autism Center for Excellence Project to actively continue our work in autism. Funding from the Simons Center for the Social Brain and the Bertarelli Foundation supports the development of accelerated diffusion and functional MRI scans with real-time motion tracking and development of an optimized data analysis pipeline. This will enable us to better understand the nature of disrupted connectivity in autism and its relation to core symptoms. The state-of-the-art system we are developing allows for the scanning children with autism without requiring sedation or anesthesia by incorporating millisecond level motion detection and correction into faster pulse sequences.

Additionally, we secured funding from the National Institute of Health - Autism Center for Excellence Program to address the mechanisms and treatment of the approximately 30% of children with ASD who remain minimally verbal in the school years. As part of this large-scale, multidisciplinary and multisite project, we running diffusion and functional MRI scans on (1) ASD adolescents (ages 14-21) with varying levels of speech/language abilities and (2) minimally-verbal ASD children (ages 6-10) before and after intensive therapy to increase speech output. With this dataset, we hope to identify neurobiological markers that predict and correlate with treatment response and to illuminate the neural basis of speech/language deficits so they can be effectively treated.

KEY RESEARCH ACCOMPLISHMENTS

- Acquired phenotyping data, ASD characterization (if applicable), anatomical images, DTI images, fMRI images, and genotyping data for a total of 53 participants with ASD and 36 control participants.
- All phenotyping data and ASD characterization have been entered ino REDCap.
- Preprocessed anatomical, fMRI, and DTI data.
- Performed quality control including motion assessment.
- Presented initial findings on resting state functional connectivity in relation to RRBs at two conferences.
- Secured funding for the development of accelerated diffusion and functional MRI scans with motion tracking system for children with autism.
- Secured funding to investigate the mechanisms and treatment of minimally-verbal children with autism.

REPORTABLE OUTCOMES

Since the completion of data acquisition, we reported our initial findings at the Conference on Resting State Brain Connectivity (Sept 2014) and the Simons Center Annual Poster Session on the Social Brain (Nov 2014):

T.Q.Nguyen, B. Baran, K.R. Van Dijk, S. Santangelo, S. Whitfield-Gabrieli, D.S. Manoach. Functional Connectivity of the Dorsal Anterior Cingulate Cortex Predicts Restrictive Repetitive Behaviors in Autism Spectrum Disorder

We are in the process of writing the manuscript based on these analyses, which address our aim of identifying the neural basis of restricted, repetitive behaviors in autism. We include a summary of this work below. This is one representative project of many that Is making use of this dataset.

Introduction:

Although restricted, repetitive behaviors (RRBs) are a highly disabling core feature of Autism Spectrum Disorders (ASDs), they have received little research attention and effective treatments are lacking. RRBs manifest as early as 18 months, predict outcome independently of social and communication deficits, and may interfere with the development of social and communication skills[1,2]. RRBs include stereotyped motor mannerisms, preoccupation with non-functional objects, extreme rigidity and insistence on sameness, and circumscribed interests. The heterogeneity of RRBs has impeded efforts to understand their neural bases, which could guide efforts to develop effective treatments.

Converging lines of evidence have linked RRBs to the structure and function of the anterior cingulate cortex (ACC). RRBs in ASD phenotypically overlap with compulsions in Obsessive Compulsive Disorder (OCD), which are associated with ACC abnormalities[3,4]. In OCD, compulsions are successfully treated with cingulotomy. OCD compulsions also respond to SSRIs and drug-related improvement in symptom severity is associated with decreased functional MRI activation in the ACC. Increased ACC and insula activation correlates with severity of circumscribed interests in ASD[5].Altered functional connectivity of the dorsal ACC (dACC) during a response inhibition task correlates with RRB severity in ASD[6].

Therefore, we predicted that aberrant resting-state functional connectivity of the dACC cognitive control network would be associated with severity of RRBs in ASD. To define the cognitive control network, we used a dACC seed from a previous study of a similar age group[7].

Methods:

Participants were selected from a larger sample of 89 participants (n_{ASD}= 53, $n_{CONTROL}$=36). Excluded participants with motion parameters > 1.5 SD from the sample mean.

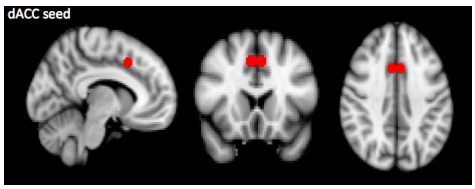
	ASD (n = 34) Mean(SD)	Control (n = 35) Mean (SD)	*p*
Age (yrs)	14.09 (3.9)	14.6 (4.6)	.64
Sex	7 F, 32 M	6 F. 29 M	
Outlier (artifact) images	14.2 (10.2)	13.2 (10.2)	.68
Head motion (mm)	.086(.099)	.087 (.067)	.91

* Motion did not correlate with ADI-R total score (r = .25, *p* = .16) or RRB severity (ADI-R C sub-score, r = .01, *p* = .9).

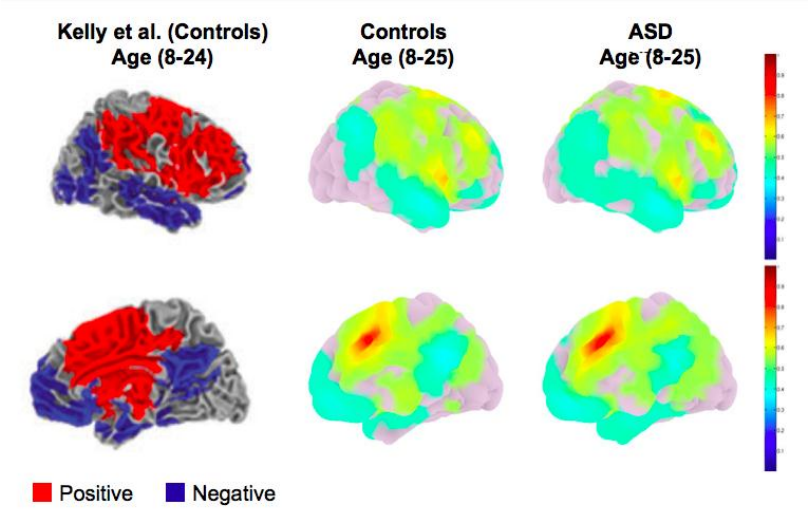
RRB Quesionnaire: Autism Diagnostic Interview – Revised (ADI-R) RRB sub-score (range: 0-12)

fMRI acquisition and analysis:

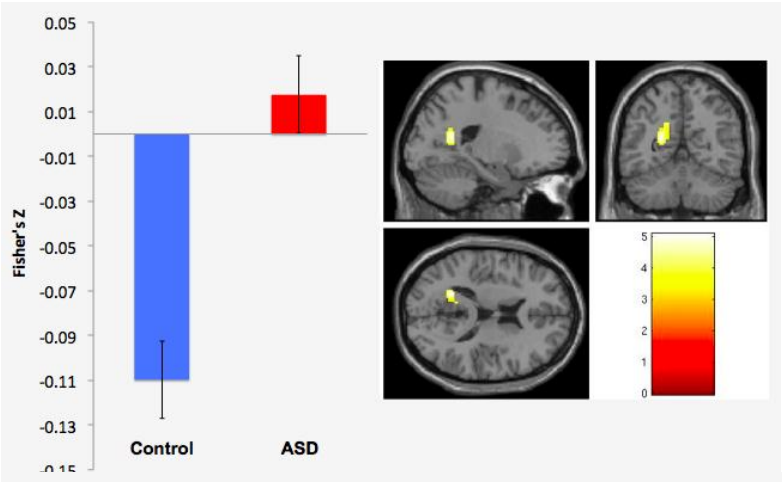
- Siemens 3T Trio; Two 6-minute resting-state fMRI with PACE
- Functional data preprocessed in SPM8
- Artifact Detection Tools (ART) excluded time-points with relative motion > 2 mm or signal intensity > 3 SD from the global mean).
- Connectivity toolbox (Conn) regressed out motion artifacts and physiological sources of noise and performed functional connectivity analyses.
- Bilateral dACC [±5, 14, 42] [7] seeds with a 6 mm diameter.
- Positive and negative connectivity of the cognitive control network compared in ASD and control groups.
- Regression of ADI-R RRB on dACC connecctivity in ASD.
- In all analyses, reported clusters survived a height threshold of uncorrected $p<0.001$ and an extent threshold of FDR-corrected $p<0.05$ at the cluster level.



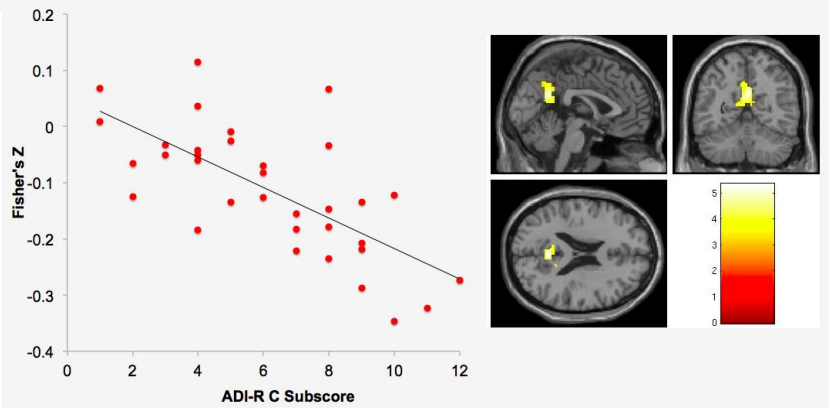
Results:
Both groups showed positive connectivity of the dACC with the 'cognitive control' network (e.g. DLPFC, VLPFC, medial and superior PFC, and IFG) and negative connectivity with regions of the 'default network' similar to a previous study (Kelly et al.[7]).



A cluster in the left medial posterior temporal lobe extending to the precuneus (peak MNI [-20, -56, 16], cluster size = 258 voxels) negatively correlated with the dACC seed in the controls but not in the ASD group.



Severity of RRBs in ASD correlated with the connectivity of the dACC seed with a region including the bilateral PCC and the left precuneus (peak MNI [-8, -46, 16]; cluster size = 558voxels). This cluster is part of network that is negatively connected to the dACC seed in the control group.



Discussion:

- As in prior studies, ASD participants moved more than their typically developing peers. Consequently, more ASD participants were excluded to match the groups for motion. In the final ASD group, motion did not correlate with the severity of RRBs.
- In both ASD and typically developing participants, the dACC cognitive control network was negatively correlated with regions of the default network.
- ASD participants showed reduced negative connectivity between dACC and the left medial posterior temporal lobe and ventral precuneus.
- Increased negative connectivity between the cognitive control network and the ventral precuneus portion of this region correlated with RRB severity.
- The ventral precuneus has been associated with self-referential processing[8].
- To better understand the relation of the connectivity of this region with RRBs we are examining what type of RRBs relate to connectivity (e.g. sensorimotor versus ideational) and will look for structural correlates of reduced functional connectivity using Diffusion Tensor Imaging.

CONCLUSION

Our final enrollment figure is 96 participants and we successfully completed data acquisition for 89 participants (53 ASD, 36 neurotypical controls). After performing qualitative and quantitative quality control and pre-processing of the data, we have been actively processing and analyzing the rich dataset from multiple perspectives. This includes investigation of how altered functional and structural connectivity in individuals with ASD is related to RRBs and speech language abilities and genetic variation. We presented our preliminary findings at the Conference on Resting State Brain Connectivity (Sept 2014) and the Simons Center Annual Poster Session on the Social Brain (Nov 2014). Using data from this project as pilot, we have secured funding from the Simons Center for the Social Brain, the Bertarelli Foundation, and the NIH - Autism Center for Excellence Project to actively continue our work in autism.

REFERENCES:

1. Morgan, L., Wetherby, A.M., & Barber, A. *J Child Psychol Psychiatry*. **49**(8), 826-37 (2008).
2. Watt, N., Wetherby, A.M., Barber, A., and Morgan, L., *J Autism Dev Disord*. **38**(8)
3. Kim, C.H., et al. *Acta Psychiatrica Scandinavica*. **107**, 283-290 (2003).
4. Rosenberg, D.R., & Keshavan, M.S. *Biol Psychiatry*. **43**(9), 623-640 (1998).
5. Cascio, C.J., et al. *J Child Psychol Psychiatry*. **55**(2), 162-171 (2014).
6. Agam, Y., Joseph, R.M., Barton, J.J.S., Manoach, D.S. *NeuroImage*. **52**, 336-347 (2010).
7. Kelly, A.M., et al. *Cerebral Cortex*. **19**, 640-657 (2009).
8. Zhang, S., Li, C.R., NeuroImage. 59, 3548-3562 (2012).